

Rejections under 35 U.S.C. §112

Claims 37-44, 46, 47 and 50 have been rejected under 35 USC §112, first paragraph, as not enabled for a cancer vaccine comprising several cell types. Applicants respectfully request clarification as to the nature of this rejection. Claim 50 is the only claim that specifically states that more than one cell type is used; is the Examiner suggesting that all claims (Claims 37-50) read on vaccines comprising more than one cell type? Applicants respectfully request clarification as to whether this basis of rejection applies to Claims 37-50 or just to Claim 50. In any event, Claim 50 depends from Claim 37, amended as discussed below. As amended, Applicants respectfully submit that Claim 50 is enabled.

Claims 37-44, 46, 47 and 50 have been rejected under 35 USC §112, first paragraph, as not enabled for a cancer vaccine for treatment of all cancers. As amended, Claims 37 now reads, "A cancer vaccine comprising tumor or other antigen presenting cells. . .". No new matter is added; the specification describes throughout that the methods of the present invention, in which fusion proteins are transferred to the surface of cells, is intended to be carried out on tumor and other antigen presenting cells.

Applicants respectfully submit that Claims 37-44, 46, 47, 49 and 50 are enabled, and that one skilled in the art would understand how to make and use the cells of the present invention in the treatment of cancer, with a reasonable expectation of success. Cancer therapy has advanced far beyond that shown in the references cited by the Examiner (published in 1994 and 1997), and one skilled in the art would be able to determine which type of tumor or other antigen presenting cell (or a combination of such cells, as in the case of Claim 50) should be selected for use in the vaccine of the present invention, depending on the type of cancer to be treated and the type of fusion protein to be transferred. Moreover, as pointed out by the CAFC in *In re Wands*, some experimentation is acceptable. One skilled in the art could easily screen various tumor or other antigen presenting cells or combinations of cells having transferred fusion proteins for their ability to evoke an immune response and reduce or eliminate tumors *in vivo*; such testing is routine and not undue under the standard set forth in *In re Wands*. Claims 37-44, 46, 47, 49 and 50 relate to treatment of cancer only, not to treatment of autoimmune, alloimmune or other diseases, as suggested by the Examiner in the Office Action. Applicants respectfully

submit that claims 37-44, 46, 47, 49 and 50 are enabled and request that this basis of rejection be withdrawn.

Claims 23 and 37 were rejected under 35 USC §112, second paragraph, as vague and indefinite. Applicants respectfully submit that as amended, these claims clearly identify the protein to be transferred, that is, the fusion protein. Applicants respectfully request withdrawal of this basis of rejection.


Claims 44 and 46 have been rejected under 35 USC §112, second paragraph, as indefinite for use of the term "costimulator"; according to the Examiner it is unclear what molecule is to be costimulated. Applicants respectfully submit that it is not a molecule that is being stimulated; it is the target cells, T-lymphocytes, which require costimulation for clonal expansion to occur. It is widely known that costimulation is provided by one or more cell surface molecules expressed by APCs; see, for example, US Patent 6,444,792, which describes the costimulatory function of several molecules such as B7-1, B7-2, and others. Applicants submit that this term is not indefinite and request withdrawal of this basis of rejection.

Claims 45 and 48 have been cancelled, thus obviating the §112, second paragraph rejection as it pertains to these claims. However, insofar as this rejection may be relevant to Claims 58 and 61, Applicants respectfully submit that the term "inhibitor" is well known in the art and is used to denote those molecules expressed on the surface of APCs which are known to suppress T-cell activation and proliferation. Examples include the inhibitory molecules listed in Claim 61. Use of this term in Claims 58 and 61 does not render these claims indefinite.

#### SUMMARY

As all outstanding issues have been addressed, it is respectfully submitted that Claims 23, 37-44, 46, 47, 49 and 50-61 are in condition for allowance; such action is respectfully requested at an early date.

Respectfully submitted,

  
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**APPENDIX**

**Deletions are depicted by underlining; additions by strikethrough.**

**In the specification:****Page 10, third full paragraph, which wraps to page 11:**

In a first set of optimization experiments, efficient incorporation of pal-prot A was documented in four cell lines (Fig. 1A) as detected with FITC-conjugated human IgG. As a negative control, nonderivatized protein A lacked the capacity to bind to the same cells. Data from the FACStar® flow cytometer analysis was plotted as arbitrary units of log10 fluorescence intensity versus number of EL-4 cells; membrane incorporation was dose dependent and started to plateau at about 33 µg/ml pal-prot A, as shown in Fig. 1B. EL-4 cells were incubated with 33 µg/ml pal-prot A for the indicated periods of time and processed as above; pal-prot A incorporation was rapid, appearing immediately after addition to the cells and reaching a plateau at ~ 1 h, as shown in Fig. 1C. This data demonstrates that numerous different cell lines can be used in the present protein transfer methods, and that the lipidated protein was incorporated into the cell fairly rapidly.

**Page 11, last paragraph:**

Cells precoated with pal-prot A were washed once and resuspended in RPMI 1640 medium ( $3-7 \times 10^6$  cells/ml). pREP7B-transfected K562 cells (K562/REP7b) were serially incubated with 33 µg/ml protein A for 2 h, 33 µg/ml Fcγ<sub>1</sub> fusion protein for 1 h, and BB-1 as primary Ab and FITC-conjugated goat anti-mouse IgG as secondary Ab. To monitor protein delivery,  $10^6$  cells were washed twice with the same buffer as above, incubated on ice for 1 h with 1 µg of human B7-specific mAb BB-1 (PharMingen, San Diego, CA) in 100 µl of buffer. Cells were washed once and immunostained (on ice for 1 h) with 100 µl of 1:100 diluted FITC-conjugated goat F(ab')<sub>2</sub> anti-mouse Ig (Boehringer Mannheim, Indianapolis, IN) as secondary Ab. Cells were washed once, resuspended in PBS, and analyzed on a FACStar® flow cytometer.

**Page 13, second full paragraph**

PBMC were isolated from fresh whole blood by Ficoll density centrifugation. T-cells were purified by two rounds of treatment with Lympho-kwik (One Lambda, Canoga Park, CA). T-cell purity was verified by lack of a proliferative response to phytohemagglutinin ("PHA") or ~~{???~~ PMA in the absence of accessory cells. The human

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CD3-specific mAb HIT3a (PharMingen) was bound to 96-well plates at the indicated concentrations and used in this form to provide a first activating signal to T-cells.

Alternatively, PHA was used in soluble form as a source of a first signal. K562 cells transfected with the negative control vector pREP7 $\beta$  (K562/pREP7 $\beta$ ) were precoated with pal-prot A and secondarily coated with B7-1·Fc $\gamma_1$ . For each proliferation assay,  $1 \times 10^5$  T-cells were incubated with  $4 \times 10^4$  B7-1·Fc $\gamma_1$ -coated and mitomycin C-treated K562/REP7 $\beta$  cells for 60 h at 37° C. Wells were pulsed with 1  $\mu$ Ci [ $^3$ H]thymidine for the last 16 h of the incubation period. Cells were harvested and counted on a Betaplate liquid scintillation counter.

**Page 18, the paragraph under subheading "Example 4"**

DBA/2J mice were purchased from The Jackson Laboratory, Maine. The animals were inoculated intradermally with a ~~lethal~~-lethal dose of L5178Y-R tumor cells and given subcutaneous injections of a cell vaccine as a treatment on days 5, 6, and 7 after the tumor inoculation. The same cell vaccine in Example 3 was used here, at a dose of  $10^6$  cells per injection. Figure 10 shows that the cell vaccine improved the survival rate of the treated animals. In Fig. 10: open circle, an untreated control group (n=8); square, another control group that received a control vaccine generated by protein A transfer (n=8); closed circle, the test group that received the cell vaccine generated by protein transfer with the immune costimulatory fusion proteins in complex with lipidate protein A (n=8).

**In the claims:**

23. A cell having a transferred fusion protein, said fusion protein transferred by:

- coating the surface of said cell with a first protein, wherein said first protein is a lipidated protein; and
- contacting said cell with a second protein, wherein said second protein is a said fusion protein and is comprised of a first domain having affinity for said first protein and a second domain having *trans* signaling and/or adhesion function.

37. A cancer vaccine comprising:

- Tumor or other antigen presenting cells having a transferred fusion protein, said fusion protein transferred by coating the surface of said cell with a first protein, wherein said first protein is a lipidated protein; and

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contacting said cells with a second protein, wherein said second protein is a said fusion protein and is comprised of a first domain having affinity for said first protein and a second domain having *trans* signaling and/or adhesion function, said cells in a suitable carrier.

**Please cancel Claims 45 and 48.**

**Please add the following new claims:**

51. The cell of Claim 23, wherein said first protein is selected from the group consisting of lipidated protein A and lipidated protein G.

52. The cell of Claim 23, wherein said first protein is palmitated protein A.

53. The cell of Claim 23, wherein said first domain is attached at the amino terminus of said second protein.

54. The cell of Claim 23, wherein said first domain is attached at the carboxyl terminus of said second protein.

55. The cell of Claim 23, wherein said second domain encodes a type I membrane protein.

56. The cell of Claim 23, wherein said second domain encodes a type II membrane protein.

57. The cell of Claim 23, wherein said second domain encodes a costimulator.

58. The cell of Claim 23, wherein said second domain encodes an inhibitor.

59. The cell of Claim 57, wherein said costimulator is selected from the group consisting of B7-1, B7-2, ICAM-1, ICAM-2, ICAM-3, CD48, LFA-3, 4-1BB ligand, CD30 ligand, CD40 ligand, and heat stable antigen.

60. The cell of Claim 59, wherein said second protein is B7-1·Fcγ<sub>1</sub>.

61. The cell of Claim 58, wherein said inhibitor is selected from the group consisting of CD8, Fas ligand and a single chain Fv derivative of immunoglobulin.